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A QUANTITATIVE STUDY OF THE DEOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID IN THE LIVERS OF ALBINO MICE DURING THE ESTRUS CYCLE

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CHAPTER I

INTRODUCTION

In the continuing attempt to understand carcinogenesis, any changes which occur during the induction of a neoplasm are of obvious interest. Hepatomas and cirrhosis of the liver have been induced by oral and intraperitoneal administration of carbon tetrachloride. Several workers showed definite changes in the amount of deoxyribonucleic acid and ribonucleic acid present in such abnormal livers.[1,2,3] Dudley, Coppock, and Johnson demonstrated a difference in the nucleic acid content between normal livers and the livers of mice fed carbon tetrachloride.[4]

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[1] R. E. Stowell, C. S. Lee, K. K. Tsuboi, and A. Villasana, "Histochemical and Microchemical Changes in Experimental Cirrhosis and Hepatoma Formation in Mice by Carbon Tetrachloride," Cancer Research, XI (May 1951), 352-353.

[2] K. K. Tsuboi, R. E. Stowell, and C. S. Lee, "Chemical Alterations Induced in Mouse Liver Following a Single Feeding of Carbon Tetrachloride," Cancer Research, XI (January 1951), 92.

[3] L. J. Carlson, D. J. Johns, R. F. Morrison, L. D. Spriggs, and E. F. Suters, "DNA, RNA, Lipid Phosphorus, and Acid-Soluble Phosphorus in Livers of A-Jax Mice Fed Carbon Tetrachloride," (1960), Unpublished data, Drake University Biology Department.

[4] D. S. Dudley, W. H. Coppock, and L. P. Johnson, "DNA, RNA, Lipid Phosphorus, and Acid-Soluble Phosphorus in Normal A-Jax Mouse Livers," Proceedings of the Iowa Academy of Science, LXVI (1959), 430.

However, this difference was obscured by the variation within the experimental and control groups.

Several factors, such as age, enzymes, and hormones, might cause a variation in the amount of deoxyribonucleic acid and ribonucleic acid present in the livers of normal mice. Until these variables have been investigated, interpretation of the data pertaining to the effects of carcinogenic agents on nucleic acid content will be difficult.

Spriggs found that the nucleic acid content of mouse liver varied according to age, and he reported that a cyclic phenomenon suggestive of the estrus cycle was evident in his data.[1] It was, therefore, the purpose of this investigation to determine the amount of nucleic acids present in the livers of albino mice during the various stages of the estrus cycle.

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[1]Lacey D. Spriggs, "A Quantitative Study of Nucleic Acids in the Liver Tissue of A/Jax Mice," (unpublished Master's thesis, Drake University, Des Moines, 1963).

CHAPTER II

HISTORY

Carbon tetrachloride, a carcinogen, has been shown to cause hepatomas and cirrhosis of the liver in mice and rats. Several investigators have found that cirrhotic livers exhibit much variation in nucleic acid content. Tsuboi, Stowell, and Lee have demonstrated that a single feeding of carbon tetrachloride caused an initial decrease followed by a rapid increase in the nucleic acid content of mouse liver.[1] Bi-weekly feedings have yielded similar results.[2] Carlson, Johns, Morrison, Spriggs, and Suters have shown that continual feedings of carbon tetrachloride, at least up to twenty-seven weeks, produced changes in the amount of nucleic acids present in mouse livers.[3] The greatest amount of deoxyribonucleic acid was present after the sixth feeding, while the ribonucleic acid reached peaks after the sixth, eighteenth, thirty-sixth, and forty-second feedings. The fluctuation of ribonucleic acid exceeded that of deoxyribonucleic acid in each of the studies involving carbon tetrachloride.

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[1] Tsuboi, *et al.*, *loc. cit.*

[2] Stowell, *et al.*, *loc. cit.*

[3] Carlson, *et al.*, *loc. cit.*

Considerable variation in the quantity of nucleic acids in normal mouse liver has also been reported. Dudley, Coppock, and Johnson have demonstrated a wide variation in the quantity of nucleic acids present in the livers of normal mice.[1] Spriggs reported that age affected the nucleic acid content of normal mouse liver.[2] Spriggs also found some interesting cyclic patterns and he suggested that these patterns might coincide with the estrus cycle.

The hormones, estrogen and progesterone, which are secreted in various quantities during the estrus cycle, have been shown to have a mitogenic effect on the reproductive organs.[3,4] Several investigators have demonstrated that progesterone caused the mitotic division of uterine muscle cells and that estrogen was more effective in epithelial and connective tissue cells of the genital tract.[5,6]

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[1]Dudley, et al., loc. cit.

[2]Spriggs, loc. cit.

[3]William S. Bullough, "Mitotic Activity in the Adult Female Mouse, Mus musculus L. A Study of Its Relation to the Oestrous Cycle in Normal and Abnormal Conditions," Proceedings of the Royal Society of London, CCXXXI (May 1946), 510.

[4]William S. Bullough, "Stress and Epidermal Mitotic Activity. II. The Effects of the Sex Hormones," Journal of Endocrinology, VIII (October 1952), 375.

[5]W. R. Crandall, "Effect of Progesterone on Cell Division in Circular Muscle of Rabbit's Uterus," Anatomical Record, LXXII (October 1938), 208.

[6]Ethel Burack, J. M. Wolfe, and A. W. Wright, "Effects of the Administration of Estrogen on the Connective Tissue of the Genital Tract of the Rat," Endocrinology, XXX (February, 1942), 343.

Jeener and Telfer demonstrated that the ribonucleic acid content in the uterus of the mouse and the rat, respectively, was responsive to the estrogens.[1,2] Villee confirmed these results by demonstrating that ovariectomy caused a decrease in the amount of ribonucleic acid in the uteri of rats and mice; and he showed that the nucleic acid content was rapidly restored to normal by the injection of estradiol.[3] Volkaer, Gompel, and Ghilain have shown that the deoxyribonucleic acid content of the human endometrium and vaginal epithelial cells varied with the stage of the menstrual cycle, reaching its peak at ovulation.[4]

Various effects on liver cells have been ascribed to gonadal hormones. Allan demonstrated that estrogen increased the number of binucleate liver cells in rabbits, but Bullough showed that esterone did not increase the number of mitoses

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[1]R. Jeener, "Acides Nucleiques et Phosphatases Au Cours De Phenomenes De Croissance Provoques Par L' Oestradiol et La Prolactine," Biochima et Biophysica Acta, II (October 1948), 453.

[2]Mary A. Telfer, "Influence of Estradiol on Nucleic Acids, Respiratory Enzymes, and the Distribution of Nitrogen in the Rat Uterus," Archives of Biochemistry, XLIV (May 1953), 118-119.

[3]Claude A. Villee, "Some Current Speculations on the Action of Estrogens," Perspectives in Biology and Medicine, II (Spring 1959), 295.

[4]R. Volkaer, C. Gompel, and A. Ghilain, "Variations in the Content of DNA in the Human Uterine and Vaginal Receptors During the Menstrual Cycle," Nature, CLXXII (July 1953), 31-32.

in mouse liver cells.[1,2] Bond described a protein found in the liver of only male rats.[3] Bond suppressed this protein by castration and restored it by the administration of testosterone. Although Common, Chapman, and Maw reported that gonadal hormones increased the ribonucleic acid and the deoxyribonucleic acid content in fowl liver, no such effect is described for mammalian liver.[4]

The correlation of any variable with the estrus cycle requires that the position in that cycle be accurately known; and the study is improved if the cycles are consistent. In practice, both of these desirables are not always present.

The estrus cycle has been studied extensively in all domestic animals and in most wild animals. All of the earlier investigators who studied mammalian reproductive cycles utilized external signs, such as the size of the

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[1]John C. Allan, "Quantitative Study of the Effects of Estradiol Benzoate and Progesterone in Modifying the Incidence of Binucleated Cells in the Rabbit Liver," Endocrinology, XXXIV (January 1944), 57.

[2]William S. Bullough, "Mitotic Activity in the Adult Female Mouse, Mus musculus L. A Study of Its Relation to the Oestrous Cycle in Normal and Abnormal Conditions," Proceedings of the Royal Society of London, CCXXXI (May 1946), 515.

[3]H. E. Bond, "The Occurrence of a Sex-Associated Protein in the Liver Tissue of the Male Rat," American Zoologist, I (March 1961), 344.

[4]R. H. Common, D. G. Chapman, and W. A. Maw, "The Effect of Gonadal Hormones on the Nucleic Acid Content of Liver and Serum in the Immature Pullet, and the Difference Between the Nucleic Acid Content of the Livers of Sexually Mature Pullets and Cockerels," Canadian Journal of Zoology, XXIX (August 1951), 273-275.

vulva, condition of the vaginal mucosa, gaping of the vaginal orifice, and the "copulatory response", none of which were very reliable. An examination of the vaginal contents has become the most successful method for studying reproductive cycles. Allen was the first investigator to study the estrus cycle in mice by vaginal smears, using a technique which was demonstrated on guinea pigs by Stockard and Papanicolaou.[1,2]

The estrus cycle in mice may be altered by environmental conditions. Several workers have reported abnormal cycles in field mice as a result of varied lighting.[3,4] Other investigators demonstrated that domestic and laboratory animals exhibit no changes in their reproductive

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[1]Edgar Allen, "The Oestrous Cycle in the Mouse," American Journal of Anatomy, XXX (May 1922), 344-348.

[2]Charles R. Stockard and G. N. Papanicolaou, "The Existence of a Typical Oestrous Cycle in the Guinea Pig With a Study of Its Histological and Physiological Changes," American Journal of Anatomy, XXII (September 1917), 280-282.

[3]John R. Baker and R. M. Ranson, "Factors Affecting the Breeding of the Field Mouse (Microtus agrestis) I. Light," Proceedings of the Royal Society of London, CX (March 1932), 321.

[4]F. H. A. Marshall and F. P. Bowden, "The Effect of Irradiation with Different Wave-Lengths on the Oestrous Cycle of the Ferret, With Remarks on the Factors Controlling Sexual Periodicity," Journal of Experimental Biology, XI (July 1934), 421-422.

cycles as a result of seasonal changes or varied day length.[1,2] Bullough found that temperature did not play an important role in affecting the estrus cycles of birds and mammals.[3] Whitten demonstrated that the estrus cycle of mice can be changed by the absence of a male or the presence of too many females.[4,5] In the absence of a male the female mice remained in the quiescent stage. The presence of large groups of females resulted in very irregular cycles and usually terminated in anestrus.

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[1]F. H. A. Marshall, "Exteroceptive Factors in Sexual Periodicity," Biological Reviews of the Cambridge Philosophical Society, XVII (January 1942), 89-90.

[2]William S. Bullough, Vertebrate Reproductive Cycles (New York: John Wiley and Sons, Inc., 1961), 28-30.

[3]Bullough, op. cit., 39.

[4]W. K. Whitten, "Modification of the Oestrous Cycle of the Mouse by External Stimuli Associated With the Male," Journal of Endocrinology, XIII (July 1956), 402-404.

[5]W. K. Whitten, "Occurrence of Anoestrous in Mice Caged in Groups," Journal of Endocrinology, XVIII (January 1959), 106.

CHAPTER III

MATERIALS AND METHODS

The albino mice used in this investigation were obtained from a mixed stock maintained by the biology department at Drake University. Purina Laboratory Chow and water were available to the mice at all times.

Suckling mice were weaned at one month and were placed in specially constructed cages to prevent abnormal estrus cycles, specifically known as the "Whitten effect."[1] The special cages were made by partitioning a standard metal mouse cage into two compartments with one-quarter inch hardware cloth. One mature male mouse was housed in the smaller compartment (eight centimeters by nineteen centimeters) and two to four experimental female mice were placed in the larger compartment (seventeen centimeters by nineteen centimeters). Constant light obtained from two forty watt fluorescent lights (Westinghouse), located directly above the cages, was maintained throughout the investigation. This not only stabilized the estrus cycle, but also hastened the maturation of the mice.[2]

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[1] W. K. Whitten, "Modification of the Oestrous Cycle of the Mouse by External Stimuli Associated With the Male," Journal of Endocrinology, XIII (July 1956), 402-404.

[2] L. Zacharias and R. J. Wurtman, "Blindness: Its Relation to Age of Menarche," Science, CXLIV (May 1964), 1154-1155.

The experimental mice were sacrificed at the age of nine weeks. This was an attempt to eliminate any discrepancy due to age, since Spriggs had shown that age affected the nucleic acid content of mouse liver.[1] Thirty-two mice, eight at each of the four different stages of estrus, were studied. All food was removed from the cages twenty-four hours before the mice were sacrificed.

A modified vaginal smear technique was used to determine the stage of estrus.[2] A medicine dropper with a fine point (one millimeter in diameter) was rinsed in seventy per cent ethanol and then in distilled water before each examination. The medicine dropper was filled with approximately one-half milliliter of nine-tenths per cent physiological saline and was inserted into the vagina. The saline solution was expelled into the vagina and immediately was withdrawn back into the dropper. The contents of the dropper were placed on a clean standard microscope slide. One drop of aqueous methylene blue (1/10,000 - CI 922) was added to the slide and was mixed with the vaginal contents to facilitate the identification of the cells. A cover slip (twenty-two millimeters by twenty-two millimeters by one millimeter), coated with vaseline to prevent evaporation,

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[1]Spriggs, op. cit., 17-27.

[2]Allen, op. cit., 342.

was placed over the preparation.

The slides were examined with a 43X objective and a 10X ocular using a light microscope (Spencer) with a mechanical stage. The light source for the microscope was a one hundred and ten watt illuminator (American Optical No. 137). The cells present in the smear were tallied on a manual counter (Denominator Company). The number of cells in the vaginal smear was determined by selecting three rnadom areas. Each area had a width equal to the diameter of the field of vision under high power magnification (43X) and a length equal to the length of the cover slip. The stage of the estrus cycle was determined as follows:

	Cornified	Epithelial	Leucocytes
Proestrus	60-90%	5-30%	5-30%
Estrus	96-100	0-5	0-2
Metestrus	50-90	0-5	5-50
Diestrus	2-15	5-10	80-90

The mice were weighed to the nearest gram on a triple-beam balance (Sargent) and were killed by cervical dislocation. The abdominal cavity was opened and the entire liver was removed and was weighed on a precision balance (Federal Pacific Electric Company). The liver was placed into a ten milliliter glass homogenizing tube (Arthur H. Thomas Company), which was immersed in crushed

ice. Distilled water was added to the liver to make a twenty per cent homogenate. This mixture was ground for approximately three minutes in the homogenizer until the homogenate was of uniform consistency.

The nucleic acids of the mouse liver were extracted by a slight modification of Schneider's technique.[1,2] Three one milliliter samples of the liver homogenate were placed into three test tubes. Two and one-half milliliters of cold ten per cent trichloroacetic acid were added to the test tubes and the mixture was shaken for two minutes. The samples were centrifuged for five minutes in a portable centrifuge (Wilkins and Anderson). The supernatant fluid was removed with a medicine dropper and was discarded. Two and one-half milliliters of cold ten per cent trichloroacetic acid were added to the test tubes and the procedure was repeated. The second extraction completed the removal of the acid-soluble phosphate fraction.

The phospholipid fraction was extracted with ethanol. Five milliliters of seventy-six per cent ethanol were added

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[1]Walter C. Schneider, "Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Deoxypentose Nucleic Acid and of Pentose Nucleic Acid," Journal of Biological Chemistry, CLXI (November 1945), 295-300.

[2]Spriggs, op. cit., 10-13.

to the sample residue which remained after the removal of the acid-soluble phosphate fraction. The mixture was shaken for two minutes and was centrifuged for five minutes. The supernatant fluid was removed with a medicine dropper and was discarded. The residue from this extraction was treated similarly with five milliliters of ninety-five per cent ethanol.

The deoxyribonucleic acid and ribonucleic acid fraction was separated from the phosphoprotein fraction by a series of extractions with five per cent trichloroacetic acid. Two and one-half milliliters of cold five per cent trichloroacetic acid were added to the sample residue which remained after the removal of the phospholipid fraction. The mixture was shaken for two minutes and was centrifuged for five minutes. The supernatant fluid was removed with a medicine dropper and was placed into a ten milliliter volumetric flask. Five milliliters of five per cent trichloroacetic acid were added to the residue from the preceeding extraction and the mixture was heated in a water bath (Precision Porta-Temp) at ninety degrees centigrade for twenty minutes. The test tubes were removed from the water bath and were placed in cold running water until the samples were cool. The samples were shaken for two minutes and were centrifuged for five minutes. The supernatant fluid was removed with a medicine dropper and was placed

into the volumetric flask with the previous extraction. To the residue two milliliters of five per cent trichloroacetic acid (room temperature) were added and the mixture was shaken for two minutes, then centrifuged for fifteen minutes. The supernatant fluid was removed with a medicine dropper and was combined with the two previous supernatant fluids in the volumetric flask. The supernatant fraction in the flask was diluted to volume with five per cent trichloroacetic acid.

The quantity of deoxyribonucleic acid present in the liver tissue was determined by comparing the nucleic acid extract from the volumetric flasks to a standard deoxyribonucleic acid solution. Three milliliters of the contents from the volumetric flask were placed into each of three test tubes. Three milliliters of standard deoxyribonucleic acid solution (.012 per cent w/v - General Biochemicals, Incorporated, Control 5268) were placed into another test tube. As a reference, three milliliters of five per cent trichloroacetic acid were placed into a test tube. Six milliliters of diphenylamine indicator solution (one per cent w/v - Fisher Scientific Company, Lot 741001) were added to each of the test tubes. The test tubes were shaken for one minute, were heated in a boiling water bath for three minutes, and then were placed in the dark overnight. The optical density of the samples was read the

following morning, about twenty hours later, on a spectrophotometer (Beckman Double-Beam) at six hundred millimicrons.

The quantity of ribonucleic acid present in the liver tissue was determined by comparing the nucleic acid extract from the volumetric flasks to a standard ribonucleic acid solution. Three milliliters of the contents from the volumetric flasks were placed into each of three test tubes. Three milliliters of standard ribonucleic acid solution (.016 per cent w/v - General Biochemicals, Incorporated, Control 5004) were placed into another test tube. Three milliliters of five per cent trichloroacetic acid were placed into a test tube as a reference. Three milliliters of five per cent trichloroacetic acid and six milliliters of orcinol indicator solution (.2 per cent w/v - 1,3 dihydroxy-5-methylbenzene - Fisher Scientific Company) were added to each of the test tubes. The test tubes were shaken for one minute and were heated in a boiling water bath for twenty minutes. The test tubes were placed in cold running water until the contents were cool; then the optical density (O.D.) of the samples was read on a spectrophotometer at six hundred and twenty millimicrons.

The following formulae were used to calculate the quantity of nucleic acids in the liver tissue:

$$\frac{\text{mg DNA}}{\text{100 gm tissue}} = \frac{\text{O.D. Sample}}{\text{O.D. Standard}} \times \frac{\text{12 mg DNA}}{\text{100 ml soln}} \times \frac{\text{10 ml soln}}{\text{.197 gm tissue}}$$

$$\frac{\text{mg RNA}}{\text{100 gm tissue}} = \frac{\text{O.D. Sample—Corr factor}}{\text{O.D. Standard}} \times \frac{\text{16 mg RNA}}{\text{100 ml soln}} \times$$

$$\frac{\text{10 ml soln}}{\text{.197 gm tissue}}$$

In these formulae 0.197 grams of tissue was used because the serological pipette did not deliver 0.200 grams as calculated. The deoxyribonucleic acid gave some color with orcinol so a correction factor was necessary. This factor was:

$$.00405 \times 12 \times \frac{\text{O.D. DNA Sample}}{\text{O.D. DNA Standard}}$$ [1]

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[1] Spriggs, op. cit., 13.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

The nucleic acid content of the livers of the thirty-two mice sacrificed during this experiment is shown in the accompanying graph and tables. Comparisons were made among mice at the various stages of the estrus cycle and among litter mates. The "F" test, an analysis for variance, was used to determine the significance of the differences observed.[1]

The deoxyribonucleic acid content in the mouse livers is shown in Table I. The mean values at proestrus, estrus, metestrus, and diestrus were 442, 414, 447, and 400 milligrams per one hundred grams of liver tissue, respectively. Although the mean deoxyribonucleic acid content was slightly higher at proestrus and metestrus, no significant differences were found among the four stages of the estrus cycle.

The ribonucleic acid content in the mouse livers is shown in Table II. The mean values at proestrus, estrus, metestrus, and diestrus were 1010, 1029, 1033, and 1045 milligrams per one hundred grams of liver tissue respectively. Although the ribonucleic acid content increased stepwise

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[1]W. J. Youden, Statistical Methods for Chemists (New York: John Wiley and Sons, Inc., 1951), 29-31.

TABLE I

QUANTITY OF DEOXYRIBONUCLEIC ACID IN ALBINO MOUSE LIVER DURING THE ESTRUS CYCLE EXPRESSED IN MILLIGRAMS OF ACID PER ONE HUNDRED GRAMS OF LIVER TISSUE

Mouse	Proestrus	Estrus	Metestrus	Diestrus
1	455	476	362	341
2	469	421	417	350
3	392	342	465	417
4	437	384	460	381
5	392	443	432	477
6	437	381	447	487
7	474	371	483	400
8	481	490	508	348
Mean	442±30*	414±46	447±39	400±50
Standard Deviation	35	53	44	57

* - Ninety-five per cent confidence limits of the mean.

from proestrus to diestrus, no significant differences were found among the four stages of the estrus cycle.

The results from Tables I and II are summarized in Figure 1 showing the ninety-five per cent confidence limits of the mean quantity of nucleic acids. There was overlap for all stages of the estrus cycle.

TABLE II

QUANTITY OF RIBONUCLEIC ACID IN ALBINO MOUSE LIVER DURING THE ESTRUS CYCLE EXPRESSED IN MILLIGRAMS OF ACID PER ONE HUNDRED GRAMS OF LIVER TISSUE

Mouse	Proestrus	Estrus	Metestrus	Diestrus
1	1064	1072	974	1007
2	1104	1020	1118	1004
3	942	1053	988	1061
4	979	1067	980	1075
5	990	966	1085	1050
6	982	963	1026	1074
7	1050	1066	1036	1061
8	966	1028	1056	1031
Mean	1010±49*	1029±38	1033±45	1045±25
Standard Deviation	57	44	52	28

* - Ninety-five per cent confidence limits of the mean.

A statistically significant change in the deoxyribonucleic acid and ribonucleic acid content of mouse liver during the estrus cycle was not demonstrated in this investigation. Such a difference might be shown if a very large number of mice were used, but it can be inferred from this study that the difference would not be striking.

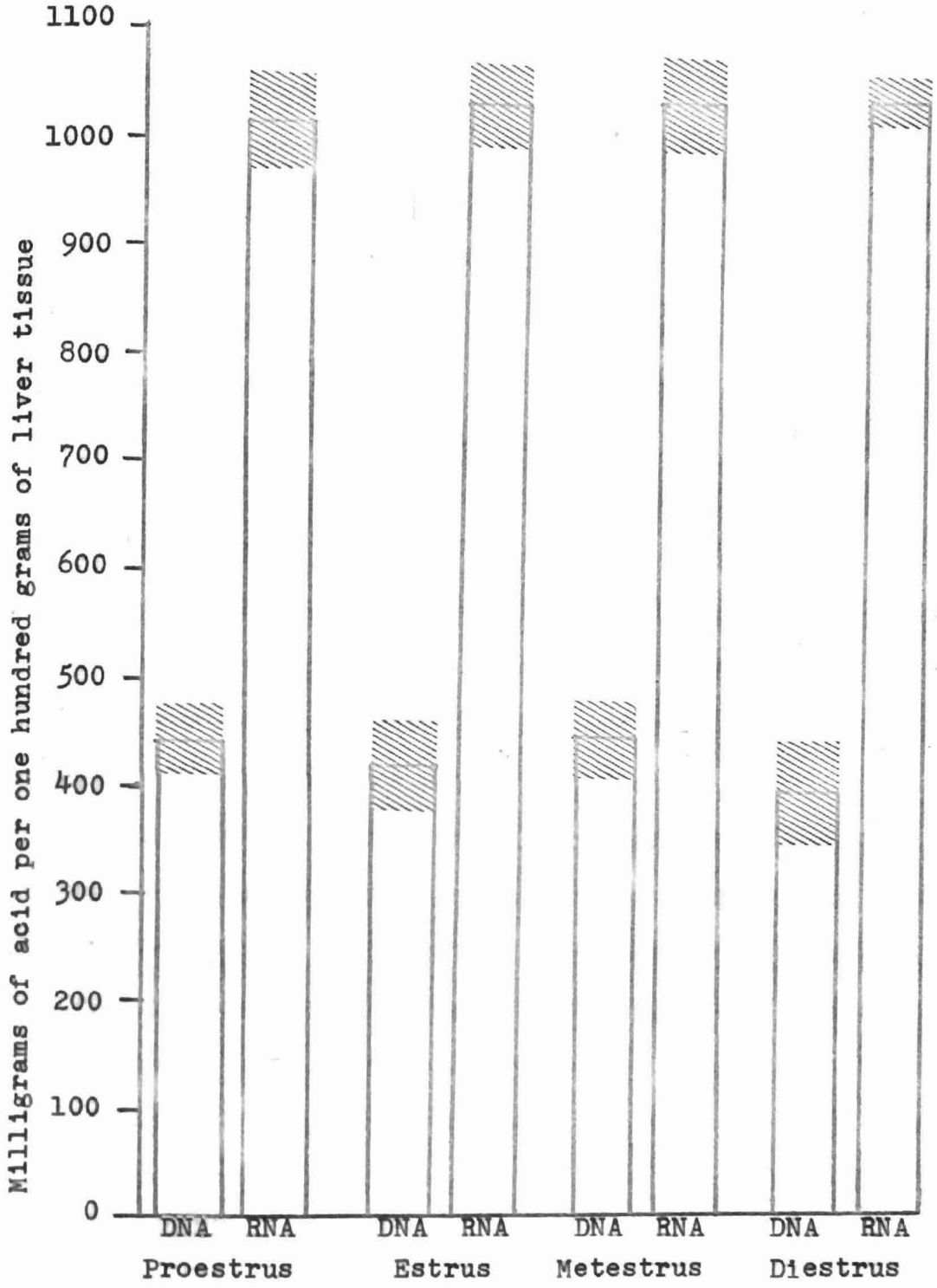

Figure 1. Quantity of deoxyribonucleic acid and ribonucleic acid in albino mouse liver during the estrus cycle.

▨ - 95% confidence limits of the mean.

It seems evident that neither the changes in the nucleic acid content of mice fed carbon tetrachloride nor the cyclic phenomenon observed by Spriggs could have been associated with the estrus cycle.[1,2,3] The changes in the nucleic acid content of mice fed carbon tetrachloride were much greater than those correlated with the estrus cycle. Spriggs' data was based on both male and female mice, which exhibited similar cyclic patterns. In his investigation no attempt was made to recognize or control the stages of the estrus cycle. Control of the estrus cycle in experimental mice is expensive, and the results of this study show that it would not reduce diversity enough to be worthwhile where deoxyribonucleic acid and ribonucleic acid content of adult livers are to be measured.

The mean differences in the nucleic acid content of mouse livers among litter mates and among mice from different litters are shown in Table III. The mean differences in the deoxyribonucleic acid and ribonucleic acid content of mouse livers among litter mates was thirty-seven and forty-six, respectively, while the mean differences among mice from different litters was fifty-three and sixty-four,

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[1]Tsuboi, et al., loc. cit.

[2]Stowell, et al., loc. cit.

[3]Spriggs, loc. cit.

TABLE III

MEAN DIFFERENCES IN DEOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID IN ALBINO MOUSE LIVER IN LITTER MATES AND NON-LITTER MATES EXPRESSED IN MILLIGRAMS OF NUCLEIC ACID PER ONE HUNDRED GRAMS OF LIVER TISSUE

Nucleic Acid	Litter mates at same stage of estrus cycle	Litter mates at different stages of estrus cycle	Non-litter mates at same stage of estrus cycle
Deoxyribonucleic acid	37	58	53
Ribonucleic acid	46	45	64

respectively. The use of litter mates is recommended in studies designed to demonstrate small changes in nucleic acid content.

CHAPTER V

SUMMARY

Many investigators have studied the deoxyribonucleic acid and ribonucleic acid content of mouse liver and some have suggested that the nucleic acid content varied with the estrus cycle. It was the purpose of this study to determine the amount of nucleic acids present in the livers of albino mice during the various stages of the estrus cycle.

Thirty-two nine week old female mice were used in this investigation. Vaginal smears were made to determine the stages of the estrus cycle. The nucleic acids in the mouse liver were extracted with trichloroacetic acid and the quantity of each acid was determined on a spectrophotometer.

No significant differences were found in the quantity of deoxyribonucleic acid or ribonucleic acid in mouse liver throughout the estrus cycle. The difference in the amount of nucleic acids present among mice from different litters was one and one-half times greater than among litter mates. It was concluded that the changes in the nucleic acid content of mouse liver did not coincide with the different stages of the estrus cycle. In future studies on the nucleic acid content of mouse liver little would be gained by controlling

the estrus cycle, but the use of litter mates would be desirable to demonstrate small differences.

BIBLIOGRAPHY

BIBLIOGRAPHY

A. BOOKS

Bullough, William S. Vertebrate Reproductive Cycles. New York: John Wiley and Sons, Inc., 1961.

Youden, W. J. Statistical Methods for Chemists. New York: John Wiley and Sons, Inc., 1951.

B. PERIODICALS

Allan, John C. "Quantitative Study of the Effects of Estradiol Benzoate and Progesterone in Modifying the Incidence of Binucleated Cells in the Rabbit Liver," Endocrinology, XXXIV (January 1944), 50-59.

Allen, Edgar. "The Oestrous Cycle in the Mouse," American Journal of Anatomy, XXX (May 1922), 297-348.

Baker, John R. and R. M. Ranson. "Factors Affecting the Breeding of the Field Mouse (Microtus agrestis) I. Light," Proceedings of the Royal Society of London, CX (March 1932), 313-322.

Bond, H. E. "The Occurrence of a Sex-Associated Protein in the Liver Tissue of the Male Rat," American Zoologist, I (March 1961), 344.

Bullough, William S. "Mitotic Activity in the Adult Female Mouse, Mus musculus L. A Study of Its Relation to the Oestrous Cycle in Normal and Abnormal Conditions," Proceedings of the Royal Society of London, CCXXXI (May 1946), 453-517.

_____. "Stress and Epidermal Mitotic Activity. II. The Effects of the Sex Hormones," Journal of Endocrinology, VIII (October 1952), 365-376.

Burack, Ethel, J. M. Wolfe, and A. W. Wright. "Effects of the Administration of Estrogen on the Connective Tissue of the Genital Tract of the Rat," Endocrinology, XXX (February 1942), 335-343.

Common, R. H., D. G. Chapman, and W. A. Maw. "The Effect of Gonadal Hormones on the Nucleic Acid Content of Liver and Serum in the Immature Pullet, and the Difference Between the Nucleic Acid Content of the Livers of Sexually Mature Pullets and Cockerels," Canadian Journal of Zoology, XXIX (August 1951), 265-275.

Crandall, W. R. "Effect of Progesterone on Cell Division in Circular Muscle of Rabbit's Uterus," Anatomical Record, LXXII (October 1938), 195-210.

Dudley, D. S., W. H. Coppock, and L. P. Johnson. "DNA, RNA, Lipid Phosphorus, and Acid-Soluble Phosphorus in Normal A-Jax Mouse Livers," Proceedings of the Iowa Academy of Science, LXVI (1959), 426-431.

Jeener, R. "Acides Nucleiques et Phosphatases Au Cours De Phenomenes De Croissance Provoques Par L' Oestradiol et La Prolactine," Biochimica et Biophysica Acta, II October 1948), 439-453.

Marshall, F. H. A. "Exteroceptive Factors in Sexual Periodicity," Biological Reviews of the Cambridge Philosophical Society, XVII (January 1942), 68-90.

_____, and F. P. Bowden. "The Effect of Irradiation with Different Wave-Lengths on the Oestrous Cycle of the Ferret, with Remarks on the Factors Controlling Sexual Periodicity," Journal of Experimental Biology, XI (July 1934), 409-422.

Schneider, Walter C. "Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Deoxypentose Nucleic Acid and of Pentose Nucleic Acid," Journal of Biological Chemistry, CLXI (November 1945), 293-303.

Stockard, Charles R. and G. N. Papanicolaou. "The Existence of a Typical Oestrous Cycle in the Guinea Pig—With a Study of Its Histological and Physiological Changes," American Journal of Anatomy, XXII (September 1917), 225-283.

Stowell, R. E., C. S. Lee, K. K. Tsuboi, and A. Villasana. "Histochemical and Microchemical Changes in Experimental Cirrhosis and Hepatoma Formation in Mice by Carbon Tetrachloride," Cancer Research, XI (May 1951), 345-354.

Telfer, Mary A. "Influence of Estradiol on Nucleic Acids, Respiratory Enzymes, and the Distribution of Nitrogen in the Rat Uterus," Archives of Biochemistry, XLIV (May 1953), 111-119.

Tsuboi, K. K., R. E. Stowell, and C. S. Lee. "Chemical Alterations Induced in Mouse Liver Following a Single Feeding of Carbon Tetrachloride," Cancer Research, XI (January 1951), 87-93.

Villee, Claude A. "Some Current Speculations on the Action of Estrogens," Perspectives in Biology and Medicine, II (Spring 1959), 290-308.

Volkaer, R., C. Gompel, and A. Ghilain. "Variations in the Content of DNA in the Human Uterine and Vaginal Receptors During the Menstrual Cycle," Nature, CLXXII (July 1953), 31-32.

Whitten, W. K. "Modification of the Oestrous Cycle of the Mouse by External Stimuli Associated with the Male," Journal of Endocrinology, XIII (July 1956), 399-404.

_____. "Occurrence of Anoestrous in Mice Caged in Groups," Journal of Endocrinology, XVIII (January 1959), 102-107.

Zacharias, Leona and Richard J. Wirtman. "Blindness: Its Relation to Age of Menarche," Science, CXLIV (May 1964), 1154-1155.

C. UNPUBLISHED WORKS

Carlson, L. J., D. J. Johns, R. F. Morrison, L. D. Spriggs, and E. F. Suters. "DNA, RNA, Lipid Phosphorus, and Acid-Soluble Phosphorus in Livers of A-Jax Mice Fed Carbon Tetrachloride," Unpublished data, Drake University Biology Department, 1960.

Spriggs, Lacey D. "A Quantitative Study of Nucleic Acids in the Liver Tissue of A/Jax Mice," Unpublished Master's thesis, Drake University, Des Moines, 1963.